Topical Review

Regulation of electromotility in the cochlear outer hair cell

Gregory I. Frolenkov

Department of Physiology, University of Kentucky, Lexington, KY, USA

Mechanosensory outer hair cells play an essential role in the amplification of sound-induced vibrations within the mammalian cochlea due to their ability to contract or elongate following changes of the intracellular potential. This unique property of outer hair cells is known as electromotility. Selective efferent innervation of these cells within the organ of Corti suggests that regulation of outer hair cell electromotility may be the primary function of the efferent control in the cochlea. A number of studies demonstrate that outer hair cell electromotility is indeed modulated by the efferent neurotransmitter, acetylcholine. The effects of acetylcholine on outer hair cells include cell hyperpolarization and a decrease of the axial stiffness, both mediated by intracellular Ca^{2+}. This article reviews these results and considers other potential mechanisms that may regulate electromotility, such as direct modification of the plasma membrane molecular motors, alteration of intracellular pressure, and modification of intracellular chloride concentration.

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Corresponding author G. Frolenkov: Department of Physiology, University of Kentucky, MS508, Chandler Medical Center, 800 Rose Street, Lexington, KY 40536, USA. Email: gregory.frolenkov@uky.edu

Inner and outer hair cells are two types of specialized mechanosensitive cells within the mammalian organ of Corti (Fig. 1*A*). Both of these cell types convert mechanical stimuli into variations of their intracellular potential (Dallos, 1992). Inner hair cells (IHCs) are the sensory cells transmitting auditory information to the brain. In contrast, outer hair cells (OHCs) receive abundant efferent innervation and have only a few afferent fibres. Although the functional significance of this efferent neural feedback for mammalian hearing is still controversial, recent studies have revealed several cellular mechanisms that may be utilized by the efferent system to control the OHC function.

OHCs are critical for the sensitivity of mammalian hearing. They generate mechanical forces for the amplification of sound-induced vibrations within the organ of Corti that are eventually perceived by IHCs (Dallos, 1992). Therefore, it is generally assumed that efferent neural feedback somehow modulates cochlear amplification by affecting OHC properties. Two different mechanisms of cochlear amplification are proposed (Fettiplace & Hackney, 2006). One mechanism is thought to be common across hair cells of all vertebrates and is based on an active mechanical feedback force generated in the sensory stereocilia of hair cells following the opening of mechanotransduction channels. The other mechanism is associated with the unique ability of cochlear OHCs to contract or elongate when the cell is depolarized or hyperpolarized, respectively (Brownell *et al.* 1985). These mechanical responses, known as 'electromotility', are driven by voltage-dependent conformational changes of prestin, a novel membrane motor protein (Zheng *et al.* 2000). Prestin populates the lateral plasma membrane of OHCs (Belyantseva *et al.* 2000*a*). Although OHCs exhibit both of these mechanisms (Fettiplace & Hackney, 2006), current data argue for a crucial role of prestin-dependent electromotility in the cochlear amplification in mammals (Liberman *et al.* 2002). This article reviews the mechanisms that regulate or may potentially regulate electromotility in OHCs.

Structural determinants of the mechanical properties of OHCs

The morphology of cochlear OHCs seems to be specifically designed for their contraction/elongation. In the organ of Corti, each OHC is mechanically coupled to the reticular lamina at the apex of the cell and to the Deiters' cell at the base, while the cylindrical cell body lacks any contacts with the adjacent cells (Fig. 1*B*). The interior of the cell is largely free of the cytoskeleton elements that are concentrated near the apex, beneath the mechanosensory stereocilia embedded into the actin-rich cuticular plate (Fig. 1*C*). The OHC body is supported by a cortical cytoskeleton that underlies the lateral plasma membrane and consists of circumferential actin filaments, cross-linked with spectrin

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(Holley & Ashmore, 1988, 1990). Circumferential stiffness of this cortical lattice is significantly higher than the axial stiffness, which determines the peculiar cylindrical shape of the OHC (Tolomeo *et al.* 1996). In addition, a potentially major contributor to OHC rigidity is the lateral plasma membrane, which has unusually large mechanical stiffness (Tolomeo *et al.* 1996). The mechanical properties of OHC plasma membrane are likely to result from the exceptionally high density of intramembrane protein particles (Gulley & Reese, 1977), which, according to the estimates of different groups, can be several thousand particles per square micrometre (He *et al.* 2006). Although it has not been proven yet, most of these particles are assumed to be prestin motors, perhaps in complex with other partner proteins.

Voltage-dependent conformations of these prestin-based membrane motors significantly affect the axial stiffness of the OHC (He *et al.* 2003). *In vivo*, when the OHC plasma membrane and the cortical cytoskeleton are under tension produced by a certain intracellular pressure (turgor), OHC axial stiffness becomes dependent on the stiffness of the cortical cytoskeleton (Adachi & Iwasa, 1997), on intracellular potential (He & Dallos, 1999), and on turgor pressure (Chan & Ulfendahl, 1997). Any one of these parameters may affect OHC mechanical properties and potentially regulate electromotility. Of course, electromotility may be also regulated by the direct modifications of the prestin-based molecular motors.

Effects of acetylcholine in OHCs

Acetylcholine is a major neurotransmitter of the medial olivocochlear efferent fibres innervating OHCs (Puel, 1995). In contrast to other potential neurotransmitters, the effects of acetylcholine on the OHCs are well documented. They are mediated by nicotinic $\alpha 9\alpha 10$ acetylcholine receptors (Elgoyhen *et al.* 1994, 2001) located at the basal pole of an OHC (Fig. 2). Application of acetylcholine to the base of an isolated OHC causes activation of these receptors (Housley & Ashmore, 1991; Housley *et al.* 1992), resulting in a brief (few milliseconds duration) inward cation current followed by a strong outward potassium current (Blanchet *et al.* 1996; Evans, 1996). The delayed potassium current is activated by Ca^{2+} entering the cell through the acetylcholine receptors (Blanchet *et al.* 1996; Evans, 1996) and is likely to be mediated by small conductance calcium-activated (SK) potassium channels (Nenov *et al.* 1996; Oliver *et al.* 2000). Without a voltage clamp, this potassium conductance is likely to hyperpolarize the OHC. The hyperpolarization would change the resting voltage sensitivity of OHC membrane motors and elongate the OHCs, in turn altering the geometry of the organ of Corti. In addition, the acetylcholine-induced conductances are expected to decrease the sound-induced receptor potential in OHCs, similar to the well-known efferent shunt effect in non-motile hair cells of lower vertebrates (Art *et al.* 1985). All these factors may affect cochlear amplification. However, *in vivo* the acetylcholine-induced

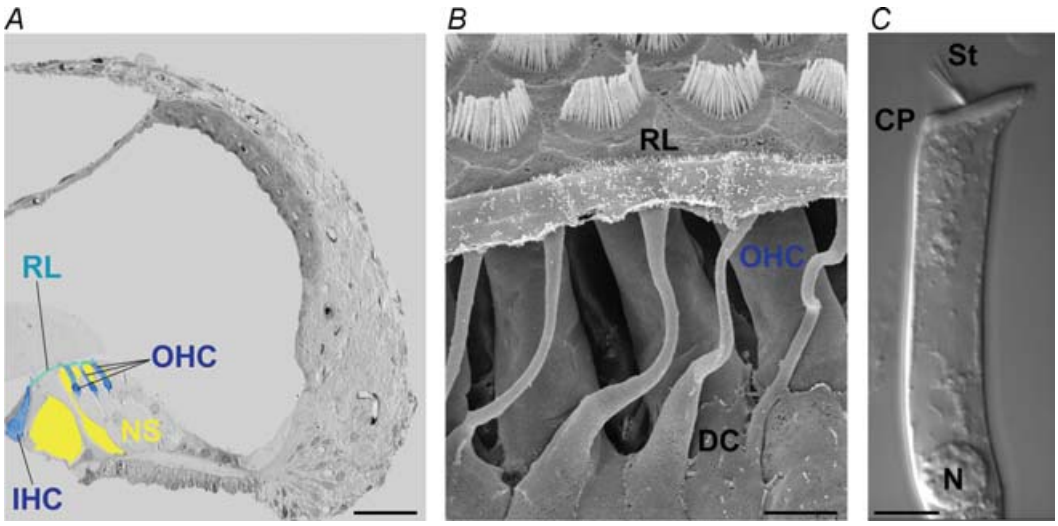

Figure 1. Outer hair cell morphology supports electromotility
A, a reconstructed image of a Lowicryl embedded section (1 μm thick) of freeze-substituted mouse organ of Corti. Outer hair cells (OHC) and inner hair cells (IHC) are coloured in blue, the reticular lamina (RL) is coloured in cyan, and Nuel space (NS) is coloured in yellow. Adapted with permission of Oxford University Press from (Ben-Yosef *et al.* 2003). *B*, scanning electron micrograph showing mechanical coupling of the guinea pig OHCs to the reticular lamina and to the Deiters' cells (DC). *C*, a live isolated guinea pig OHC observed with differential interference contrast optics. Note that most intracellular organelles are located below the cuticular plate (CP) that supports mechanosensitive stereocilia (St). A small amount of organelles is present in the middle of the cell, above the nucleus (N). Scale bars: *A*, 50 μm, *B* and *C*, 5 μm.

hyperpolarization of OHCs is likely to be moderate due to the fact that these cells already have a relatively large negative intracellular potential (Dallos, 1992).

Apart from fast activation of ion currents, acetylcholine also evokes an increase of electromotile responses that develops on a time scale of several seconds (Dallos *et al.* 1997). This increase is unlikely to be associated with the changes in the voltage-dependent conformations of the plasma membrane motor proteins (Frolenkov *et al.* 2000). Instead, acetylcholine decreases the axial stiffness of the cell and reduces the overall mechanical load of the aggregate OHC motors, resulting in the increase of electromotile responses (Dallos *et al.* 1997). Relative contributions of the cortical cytoskeleton and the plasma membrane to these acetylcholine-induced global changes of the OHC axial stiffness are still unknown. When the prestin-based motor activity is 'knocked down' by removal of intracellular chloride ions, acetylcholine is still able to reduce axial stiffness of the OHCs (He *et al.* 2003), confirming that acetylcholine-induced stiffness changes can occur independently from operation of the OHC membrane motors (Frolenkov *et al.* 2000). However, the magnitude of the acetylcholine effect is reduced without intracellular chloride (He *et al.* 2003), suggesting either the inhibition of some important intracellular signalling pathways after chloride replacement, or the existence of a prestin-dependent component in acetylcholine-induced changes of OHC axial stiffness.

The effects of acetylcholine on the OHC electromotility are Ca^{2+} dependent. First, they do not occur in a Ca^{2+}-free extracellular medium (Dallos *et al.* 1997). Second, they seem to be accompanied by a decrease of membrane-associated Ca^{2+}, which presumably reflects the release of Ca^{2+} from intracellular Ca^{2+} stores (Dallos *et al.* 1997). Third, an increase of electromotile responses can be produced by increasing intracellular Ca^{2+} with ionomycin (Frolenkov *et al.* 2000; Szonyi *et al.* 2001). Finally, ionomycin-induced changes of OHC axial stiffness are similar to the acetylcholine-induced ones (Frolenkov *et al.* 2003). Therefore, it has been suggested that acetylcholine-induced changes of the axial stiffness are mediated by Ca^{2+}-dependent phosphorylation of some components of the OHC cortical cytoskeleton (Dallos *et al.* 1997; Frolenkov *et al.* 2000; Sziklai *et al.* 2001). Meanwhile, there is a caveat to this hypothesis. In order to modify mechanical properties of the cortical cytoskeleton, the Ca^{2+} signal has to propagate from the base of the cell to the lateral wall (Fig. 2). A localized increase of intracellular Ca^{2+} following activation of the acetylcholine receptors was, indeed, demonstrated at the base of mammalian OHCs, but not in all cells exhibiting strong cholinergic current responses (Evans *et al.* 2000). Acetylcholine-induced intracellular Ca^{2+} signals may be effectively shielded from the interior of an OHC by a near-membrane endoplasmic reticulum structure, the 'synaptoplasmic' cistern (Saito, 1983). Apart from shielding the Ca^{2+} signal, the synaptoplasmic cistern amplifies it by mediating Ca^{2+}-induced Ca^{2+}-release (Evans *et al.* 2000; Lioudyno *et al.* 2004). A similar structure, the 'subsurface' cisternae (Fig. 2), is located near the lateral plasma membrane of the OHC, just beneath the cortical cytoskeleton (Saito, 1983). It is tempting to speculate that acetylcholine triggers some sort of Ca^{2+} wave propagating in proximity to the plasma membrane from the base of the cell to the lateral wall. However, this phenomenon has not yet been experimentally demonstrated. Further studies are also needed to elucidate the signalling pathways that lead to modification of the cytoskeleton by acetylcholine. Several phosphorylation pathways have been suggested to be involved in this process (Szonyi *et al.* 1999; Kalinec *et al.* 2000; Zhang *et al.* 2003).

Direct regulation of prestin

Voltage-driven conformational changes of prestin-based molecular motors are accompanied by the translocation

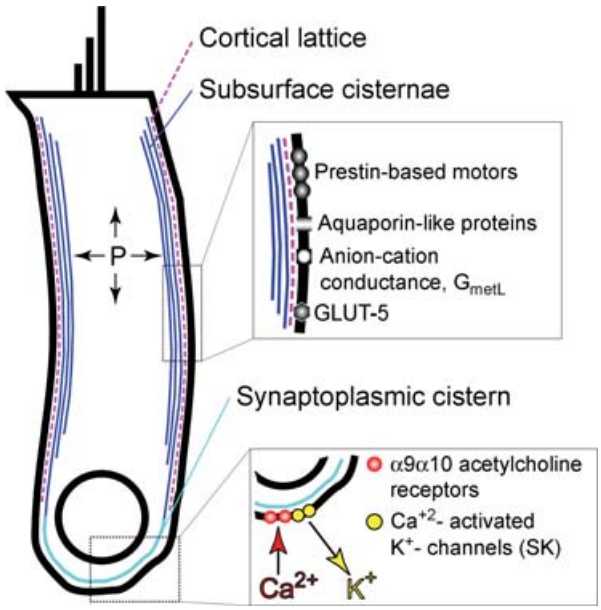

Figure 2. Cellular components potentially participating in regulation of OHC electromotility
Intracellular pressure (*P*) sets the overall tension of the plasma membrane and the cortical lattice. The lateral plasma membrane contains prestin, aquaporin-like protein, GLUT-5, and an unidentified stretch-activated channel supporting non-selective anion-cation conductance G_{metL}. Prestin molecules are shown in clusters to reflect their oligomerization and aggregation into structural and functional microdomains (He *et al.* 2006). The structural relations between prestin and other molecules in the lateral plasma membrane are unknown. Activation of nicotinic $\alpha 9\alpha 10$ acetylcholine receptors results in the inward Ca^{2+} current that activates nearby Ca^{2+}-dependent K^+ channels. This effect is amplified by Ca^{2+}-induced Ca^{2+}-release from synaptoplasmic cistern. For other details see the text.

of an electrical charge across the plasma membrane, which can be detected as voltage-dependent (nonlinear) capacitance of the OHCs (Santos-Sacchi, 1991). This nonlinear capacitance usually correlates well with voltage-driven contraction/elongation of the OHC (Santos-Sacchi, 1991). However, when the normal cylindrical shape of the OHC is disrupted by loss of turgor or when the prestin function is investigated in heterologous cells, electromotile responses are hardly detectable, but measurements of nonlinear capacitance demonstrate normal operation of prestin-based motors (Santos-Sacchi, 1991; Zheng *et al.* 2000). Furthermore, when acetylcholine and/or intracellular Ca^{2+} affects the OHC axial stiffness resulting in the increase of electromotile responses, OHC voltage-dependent capacitance also does not change (Frolenkov *et al.* 2000, 2003). In these cases, measurements of the nonlinear capacitance represent a more direct assessment of the operation of plasma membrane motor proteins than observations of OHC contraction/elongation. Using capacitance measurements, it was shown that a variety of stimuli can modify the function of the OHC motor, mostly by shifting the range of voltage sensitivity (He *et al.* 2006). It was also shown that general phosphatases or dephosphatases can modify the voltage sensitivity of the OHC motor (Frolenkov *et al.* 2000, 2001).

Cloning of the gene encoding prestin provided a wealth of opportunities to explore further potential pathways of prestin phosphorylation. It was shown that prestin has two functional cGMP-dependent phosphorylation sites, S238 and T560 (Deak *et al.* 2005). In TSA201 cells transfected with prestin cDNA, phosphorylation at these sites not only shifts the voltage sensitivity of prestin, but also increases the maximum nonlinear capacitance, a phenomenon that has not been yet observed in OHCs. If this phosphorylation pathway is functional *in vivo*, it may regulate electromotility through a direct modification of the OHC motor.

OHC turgor

Even relatively small changes of the intracellular pressure (turgor) may affect the amplitude and the operating range of electromotility (Kakehata & Santos-Sacchi, 1995; Sziklai & Dallos, 1997). In isolated OHCs, acetylcholine-induced changes of axial stiffness are not accompanied by apparent changes of cell volume (Dallos *et al.* 1997). However, subtle changes of turgor cannot be excluded even in the isolated cells (Dallos *et al.* 1997) and are certainly possible *in vivo*, where both the surroundings and the condition of the cell are likely to be different. In addition to prestin, the lateral plasma membrane of OHCs also contains an aquaporin-like protein (Belyantseva *et al.* 2000*b*) and a sugar carrier, GLUT-5 (Geleoc *et al.* 1999; Belyantseva *et al.* 2000*a*). Both water and sugar transport are voltage dependent in OHCs (Geleoc *et al.* 1999; Belyantseva *et al.* 2000*a*), suggesting an intimate interaction between these processes and the operation of the prestin-based membrane motor. Any changes of aquaporin and/or GLUT-5 permeability, for example by Ca^{2+}-dependent phosphorylation, should change water and/or sugar balance in an OHC, resulting in osmotic changes of volume and turgor, which in turn should affect electromotility. Whether or not such a hypothetical scenario is relevant to the regulation of OHC electromotility *in vivo* remains to be investigated.

Regulation by intracellular chloride

One of the most exciting recent discoveries in the field of OHC physiology is the dependence of the motor function of prestin on intracellular anions. Replacement of intracellular chloride with physiologically non-relevant anions such as pentanesulphonate or maleate completely abolishes nonlinear capacitance of prestin-containing membrane patches (Oliver *et al.* 2001) or dramatically reduces this capacitance in OHCs (Rybalchenko & Santos-Sacchi, 2003). Different groups disagree on whether a complete elimination of voltage-dependent capacitance can be achieved in anion substitution experiments (Oliver *et al.* 2001; Rybalchenko & Santos-Sacchi, 2003). Correspondingly, intracellular anions are considered as extrinsic voltage sensors (Oliver *et al.* 2001) or allosteric modulators (Rybalchenko & Santos-Sacchi, 2003) of prestin. Irrespective of the underlying molecular mechanisms, there is a general agreement that the motor function of prestin is profoundly influenced by intracellular anion species. This phenomenon opens an untouched territory for studies of electromotility regulation. Chloride transporters and channels in OHCs are not well characterized, although several voltage-dependent chloride channels were detected in OHCs by single-cell RT-PCR, including hyperpolarization-activated ClC-2 channels (Kawasaki *et al.* 1999, 2000). Furthermore, an unusual cation–anion non-selective stretch-activated conductance, G_{metL} was found on the lateral plasma membrane of OHCs (Rybalchenko & Santos-Sacchi, 2003). Manipulations of intra- and extracellular chloride concentration *in vivo* modulate sound-induced vibrations within the cochlea and can even increase the amplitude of such vibrations (Santos-Sacchi *et al.* 2006). The normal level of chloride in OHCs is near or below 10 mM, but this concentration quickly increases after OHC isolation (Santos-Sacchi *et al.* 2006). Therefore, it is very likely that all previous studies of the acetylcholine effects in OHCs were performed in the cells overloaded with chloride, especially during whole-cell patch-clamp recordings with an order

of magnitude higher chloride concentration inside the pipette. Efferent control of electromotility may be mediated by intracellular chloride through acetylcholine-induced hyperpolarization with subsequent opening of voltage-dependent chloride channels (e.g. ClC-2), through activation of Ca^{2+}-dependent chloride channels, through modulation of potassium–chloride cotransporters, and through other mechanisms. This is only the beginning of the rapidly expanding exploration of the regulatory role of chloride, and perhaps other physiologically relevant anions, in OHC physiology.

Conclusion

Some of the cellular mechanisms that regulate electromotility remain speculative while others have substantial experimental support. None of these mechanisms has yet been definitively established as a major mechanism responsible for efferent regulation of OHC function *in vivo*. Therefore, the long-standing question of how the efferent system influences cochlear function remains largely unanswered.

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